Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 6
Internal standard: niacin (3.3)

OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamethoxazole, sulfamonomethoxine

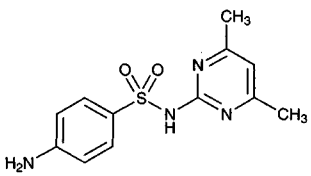
KEY WORDS

wastewater

REFERENCE

Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethazine



Molecular formula: $C_{12}H_{14}N_4O_2S$
Molecular weight: 278.33
CAS Registry No.: 57-68-1
Merck Index: 9083
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 150 μL 3% trichloroacetic acid in EtOH + 100 μL EtOH, vortex, freeze at -20° for 5 min, centrifuge, freeze at -20° for 10 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 DB
Column: 250 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:buffer 23:77 with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM

Retention time: 8
Internal standard: sulfamethazine (sulfadimidine)

OTHER SUBSTANCES
Simultaneous: sulfadiazine, trimethoprim

KEY WORDS
plasma; fish; salmon; trout; sulfamethazine is IS

REFERENCE
Hormazabal,V.; Rogstad,A. Simultaneous determination of sulfadiazine and trimethoprim in plasma and tissues of cultured fish for residual and pharmacokinetic studies, *J.Chromatogr.*, **1992**, *583*, 201–207.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut 40 μm C18 SPE cartridge with 2 mL MeOH and 2 mL pH 7.4 phosphate buffer. Add 1 mL plasma to the SPE cartridge, wash with 2 mL pH 7.4 phosphate buffer, elute with 250 μL MeOH, add 750 μL pH 6.4 phosphate buffer to the eluate, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher RP18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: MeOH:50 mM pH 6.4 phosphate buffer 25:75
Column temperature: 35
Flow rate: 0.9
Injection volume: 20
Detector: UV 262, UV 292

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 1.1 μg/mL
Limit of quantitation: 3.7 μg/mL

OTHER SUBSTANCES
Extracted: metabolites, acetylsulfamethazine

KEY WORDS
plasma; sheep; SPE; pharmacokinetics

REFERENCE
Hubert,P.; Chiap,P.; Evrard,B.; Delattre,L.; Crommen,J. Fully automated determination of sulfamethazine in ovine plasma using solid-phase extraction on disposable cartridges and liquid chromatography, *J.Chromatogr.*, **1993**, *622*, 53–60.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bakerbond C18 SPE cartridge with MeOH and 50 mM pH 5.5 citrate buffer. 500 μL Serum + 500 μL 50 mM pH 5.5 citrate buffer, vortex, add to the SPE cartridge, wash twice with 50 mM pH 5.5 citrate buffer, air dry, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 250 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:1% acetic acid 18:82
Flow rate: 2.5
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Retention time: 4.19
Internal standard: sulfamethazine

OTHER SUBSTANCES
Extracted: sulfamethoxazole, trimethoprim

KEY WORDS
serum; SPE; sulfamethazine is IS

REFERENCE
Moore,K.H.P.; Brouwer,K.L.R. High-performance liquid chromatographic evaluation of the effect of heat treatment on trimethoprim and sulfamethoxazole stability in serum, *Ther.Drug Monit.*, **1995**, *17*, 356–360.

SAMPLE
Matrix: blood, culture media, microsomal incubations, urine
Sample preparation: Plasma, urine, culture medium. 0.3 (Plasma, urine) or 2.5 (culture medium) mL sample + 1 mL 500 mM pH 4.5 acetate buffer + 20 mg limpet acetone powder (from Patella vulgata, Sigma), heat at 37° for 3 h, add 60 μL 4 M NaOH, add 1 mL 500 mM pH 6.0 phosphate buffer, add 200 μL 90 μg/mL sulfadimethoxine in MeOH:water 15:85, saturate with solid anhydrous ammonium sulfate, add 4 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5, extract, centrifuge at 500 g for 10 min, repeat the extraction with 3 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 (urine) or 0.2 (plasma) mL mobile phase, inject a 20 μL aliquot. Microsomal incubations. Microsomal incubation + 1 mL 500 mM pH 6.0 phosphate buffer + 200 μL 90 μg/mL sulfadimethoxine in MeOH:water 15:85, saturate with solid anhydrous ammonium sulfate, add 4 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5, extract, centrifuge at 500 g for 10 min, repeat the extraction with 3 mL diethyl ether:dichloromethane:isopropanol 60:40:0.5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 (urine) or 0.2 (plasma) mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm Hypersil ODS RP-C18
Column: 100 × 4 5 μm Hypersil ODS RP-C18
Mobile phase: MeOH:50 mM pH 6.67 phosphate buffer 10:90
Flow rate: 0.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 16.5
Internal standard: sulfadimethoxine (28)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; goat; rat

REFERENCE
van 't Klooster,G.A.E.; van Seeventer,P.B.; Kolker,H.J.; Smit,L.A.; Witkamp,R.F. High-performance liquid chromatographic method for the routine determination of sulphadimidine, its hydroxy metabolites and N4-acetylsulphadimidine in body fluids and cell culture media, *J.Chromatogr.*, **1991**, *571*, 157–168.

SAMPLE
Matrix: blood, eggs
Sample preparation: 1 mL Serum or 1 g homogenized egg + 4 mL MeCN, vortex, centrifuge at 3000 rpm for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 3000 rpm for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 0.01% trichloroacetic acid, centrifuge at 3000 rpm for 15 min. Remove a 500 μL aliquot and add it to 100 μL 1 mg/mL fluorescamine in MeCN (freshly prepared), shake, let stand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: chemiluminescence following post-column reaction. The column effluent was mixed with reagent pumped at 0.5 mL/min and the mixture flowed to the detector. (Reagent was 1 mM bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl] oxalate (Wako) and 300 mM hydrogen peroxide in MeCN.)

CHROMATOGRAM
Retention time: 8.1
Limit of detection: 1 ng/mL

KEY WORDS
chicken; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F.; Ueyama,Y.; Azama,J. Determination of sulfamethazine residue in chicken serum and egg by high-performance liquid chromatography with chemiluminescence detection, *J.Chromatogr.Sci.*, **1995**, *33*, 365–369.

SAMPLE
Matrix: blood, milk
Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 7.5
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfamethoxazole, sulfamonomethoxine, sulfathiazole

KEY WORDS
cow; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE
Matrix: blood, tissue
Sample preparation: 1 mL Serum or homogenized tissue + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL sulfadiazine in 0.01% trichloroacetic acid, shake, add 100

μL hexane, shake, centrifuge at 1000 g for 15 min. Remove a 500 μL aliquot of the clear aqueous layer and add it to 100 μL 1 mg/mL fluorescamine in MeCN (freshly prepared), shake by hand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pack C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 7.9
Internal standard: sulfadiazine (7.1)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfamethoxazole, sulfamonomethoxine, sulfadimethoxine

KEY WORDS
serum; pig; derivatization; kidney; muscle; liver

REFERENCE
Tsai,C.-E.; Kondo,F. A sensitive high-performance liquid chromatographic method for detecting sulfonamide residues in swine serum and tissues after fluorescamine derivatization, *J.Liq.Chromatogr.*, **1995**, *18*, 965–976.

SAMPLE
Matrix: blood, urine
Sample preparation: Blood. 320 μL Whole blood + 450 μL urea (1:1), shake mechanically for 15 min, filter (Amicon Micropartition System MPS-1) while centrifuging at 4° at 4000 rpm for at least 3 h, inject a 300 μL aliquot of the ultrafiltrate. Urine. Dilute urine if necessary. Filter (Amicon Micropartition System MPS-1) urine while centrifuging at 4° at 4000 rpm for at least 3 h, inject a 300 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 mm long 5 μm Spherisorb ODS 2
Mobile phase: MeOH:MeCN:10 mM pH 5.0 sodium acetate buffer containing 4 mM triethylamine 14:14:72
Flow rate: 0.8
Injection volume: 300
Detector: UV 275

CHROMATOGRAM
Internal standard: sulfamethazine

OTHER SUBSTANCES
Extracted: trimethoprim

KEY WORDS
fish; whole blood; trout; rainbow trout; sulfamethazine is IS

REFERENCE
Tan,W.P.; Wall,R.A. Disposition kinetics of trimethoprim in rainbow trout (*Oncorhynchus mykiss*), *Xenobiotica*, **1995**, *25*, 315–329.

SAMPLE
Matrix: blood, urine, tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL dichloromethane and dry for 15 min under a gentle stream of nitrogen. Mix 5 g minced tissue or 0.5-2 mL plasma

or 0.2-1 mL urine (adjusted to pH 6.5-7.0 with 250 mM acetic acid) with 20 mL dichloromethane, vortex for 30 s, centrifuge at 2000 g for 10 min. Filter the supernatant, dry over glass wool and anhydrous sodium sulfate, weigh the filtered extract. Add the sample to the SPE cartridge, elute with 6 mL 50 mM pH 10 phosphate buffer, collect the first 4 mL eluate, weigh, mix, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 30 μm Perisorb C8 (Chrompack)
Column: 200 × 3 5 μm Chromspher C18
Mobile phase: MeCN:pH 5.3 ammonium acetate buffer 22:78
Flow rate: 0.5
Injection volume: 100
Detector: UV 261

CHROMATOGRAM

Limit of detection: 10 ng/g (tissue), 50 ng/mL (urine, plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pig; muscle; liver; kidney; SPE

REFERENCE

Haasnoot,W.; Korsrud,G.O.; Cazemier,G.; Manevals,F.; Keukens,H.; Nouws,J. Application of an enzyme immunoassay for the determination of sulphamethazine (sulphadimidine) residues in swine urine and plasma and their use as predictors of the level in edible tissue, *Food Addit.Contam.*, **1996**, *13*, 811–822.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 200 mg amoxicillin trihydrate in 8 mL 200 mM pH 11.0 phosphate buffer, add 10 mL 70 mg/L sulfamethazine in solvent, make up to 20 mL with solvent, inject an aliquot within 1 min. Dissolve 200 mg amoxicillin sodium salt in 8 mL solvent, add 10 mL 70 mg/L sulfamethazine in solvent, make up to 20 mL with solvent, inject an aliquot within 1 min. (Solvent was MeOH:200 mM pH 7.0 potassium phosphate buffer:water 5:5:90.)

HPLC VARIABLES

Guard column: 40 × 4.6 10 μm LiChrosorb RP-2
Column: 250 × 4.6 7μm Zorbax C8
Mobile phase: Gradient. A is MeOH:200 mM pH 7.0 potassium phosphate buffer:water 5:5:90. B is MeOH:200 mM pH 7.0 potassium phosphate buffer:water 50:5:45. A:B 95:5 for 5 min, to 35:65 over 30 min, to 95:5 over 7.5 min.
Column temperature: 30
Flow rate: 1
Injection volume: 25
Detector: UV 274

CHROMATOGRAM

Retention time: 35
Internal standard: sulfamethazine

OTHER SUBSTANCES

Simultaneous: amoxicillin, impurities, amoxicilloates, amoxicillin piperazine-2,5-dione, amoxicillin dimer, amoxicillin trimer

KEY WORDS

sulfamethazine is IS

REFERENCE

De Pourcq,P.; Hoebus,J.; Roets,E.; Hoogmartens,J.; Vanderhaeghe,H. Quantitative determination of amoxicillin and its decomposition products by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *321*, 441–449.

SAMPLE

Matrix: bulk, solutions, tissue

Sample preparation: Oil. Mix 200 μL oil with 100 μL 10 ng/mL sulphamerazine and dilute with 2 mL hexane. Extract with 1 mL 10 mM hydrochloric acid. Mix 200 μL acid layer with 100 μL MeCN, inject a 25 μL aliquot. Solutions. Directly inject an aliquot, use fluorescence or UV detection. Tissue. A. 5 g Tissue + IS + 25 mL 5% acetic acid in ethyl acetate, homogenize for 1 min, centrifuge at 3000 rpm for 5 min. Repeat this procedure. Decant supernatant onto NH2 and SCX SPE cartridges in series. Discard NH2 cartridge. Wash SCX cartridge with 5 mL water, wash with 10 mL acetone, wash with 10 mL MeCN. Elute with 10 mL MeOH:ammonia 50:50. Blow-down extract to dryness, add 2 mL 10 mM hydrochloric acid. Add 100 μL 2 mg/mL fluorescamine to a 200 μL aliquot, mix well and wait for 20 min. Inject a 10 μL aliquot, use fluorescence detection. B. Homogenize tissue in dichloromethane, dry with sodium sulfate, centrifuge at 1600 rcf for 5 min. Mix supernatant with hexane, add to an activated silica SPE cartridge. Dry SPE cartridge with nitrogen, elute with MeOH. Evaporate MeOH and reconstitute with 1 mL pH 6.5 mobile phase. Inject an aliquot, use UV detection.

HPLC VARIABLES

Column: 100 × 5 4 μm NovaPak C18

Mobile phase: MeCN:buffer 20:80 (Buffer was 77 mg ammonium acetate in 800 mL water)

Flow rate: 1

Injection volume: 25

Detector: F ex 405 em 495, UV 270

CHROMATOGRAM

Retention time: 6.8

Internal standard: sulphamerazine

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

kidney; liver; muscle; pig; derivatization; SPE

REFERENCE

Rose,M.D.; Farrington,W.H.; Shearer,G. The effect of cooking on veterinary drug residues in food: 3. Sulphamethazine (sulphadimidine), *Food Addit.Contam.*, **1995**, *12*, 739–750.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 6.3
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: feed
Sample preparation: Blend 10-100 g feed with 200 mL chloroform:MeOH 75:25 for 3 min, filter through 25 mm Celite 545, re-extract residue with 200 mL chloroform:MeOH 75:25, add 50 mL chloroform:MeOH 75:25 to the residue, filter this mixture, wash the filter cake with several small portions of chloroform:MeOH 75:25. Evaporate the filtrate on a steam bath with a current of air to about 100 mL. Shake the organic layer with 50 mL 10% NaCl in 100 mM NaOH for 1 min, wash the aqueous phase with two or three 50 mL portions of chloroform (until wash is colorless), add 10 mL 1 M KH_2PO_4 solution to the aqueous phase, extract with three 50 mL portions of chloroform. Combine the extracts and filter them through a 25 mm layer of sodium sulfate, evaporate most of the filtrate on a steam bath with a current of air, evaporate the remainder with a current of air. Take up the residue in 100 mL MeOH, remove a 20 mL aliquot, evaporate most on a steam bath with a current of air, evaporate the remainder with a current of air, dissolve the residue in 100 μL MeOH, make up to 10 mL with MeCN:water 25:75, filter (0.45μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.5% acetic acid and 0.05% sodium 1-octanesulfonate.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Simultaneous: carbadox

REFERENCE

McGary,E.D. Quantitative determination of sulphamethazine and carbadox in animal feeds by paired ion high-performance liquid, *Analyst*, **1986**, *111*, 1341–1342.

SAMPLE
Matrix: feed
Sample preparation: Condition a 3 mL 500 mg cation-exchange SPE cartridge (aromatic sulfonic acid, J.T. Baker) with 10 mL MeCN:water:glacial acetic acid 70:30:20, do not allow to go dry. Grind feed to pass through a 1 mm sieve. 10 g Ground feed + 10 mL MeCN:water 35:15, shake mechanically for 1 h, filter (Whatman glass fiber GF/A). Remove a 10 mL aliquot of the filtrate and add it to 5 mL acetic acid, shake gently, add to the SPE cartridge, rinse flask with 5 mL MeCN:water:glacial acetic acid 70:30:20, add rinse to the SPE cartridge, wash with 10 mL water, wash with 10 mL MeOH, pass nitrogen through the SPE cartridge for 15 min, elute with 30 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Rosil C18 (Alltech)
Mobile phase: MeCN:buffer:water 22.5:15:62.5 (Buffer was 19.27 g ammonium acetate and 30 mL acetic acid made up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 272

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Noninterfering: acinitrazole, amprolium, arprinocid, buquinolate, carbadox, clopidol, decoquinate, diaveridine, dimetridazole, dinitolmide, ethopabate, furazolidone, halofuginone, ipronidazole, methyl benzoquate, nifursol, nitrofurazone, nitrovin, olaquindox, pyrimethamine, robenidine, ronidazole, sulfanitran, sulfaquinoxaline

KEY WORDS
SPE

REFERENCE
Conway,B. Determination of sulphadimidine in animal feeds by high-performance liquid chromatography, *Analyst*, **1988**, *113*, 1397–1400.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground feed + 100 mL solvent + 5 mL 8 μg/mL sulfamerazine in diluent, shake for 1 h, chill an aliquot in an ice bath for 2 h, centrifuge at 1650 g for 5 min, filter (0.2 μm), inject a 200 μL aliquot of the filtrate. (Prepare solvent by mixing 250 mL MeOH, 300 mL water, and 25 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water. Prepare diluent by mixing 250 mL MeOH, 300 mL water, and 12.5 mL HCl, mix, add 15 mL diethylamine, mix, make up to 1 L with water.)

HPLC VARIABLES
Guard column: C8 or C18
Column: 250 × 4.67 μm Lichrosorb RP-18
Mobile phase: MeCN:2% acetic acid 17:83
Flow rate: 1-1.3
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1 -0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm i.d. PTFE coil to the detector. (Prepare reagent by dissolving 1.5 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, mix well, add 40 mL water, mix well, prepare fresh daily.)

CHROMATOGRAM
Retention time: 8.5
Internal standard: sulfamerazine (7)
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: sulfathiazole

Simultaneous: sulfadimethoxine, sulfaquinoxaline
Noninterfering: amino acids, amprolium, apramycin, arsanilic acid, bacitracin, hygromycin B, neomycin, nystatin, ormetoprim, procaine

KEY WORDS
post-column reaction

REFERENCE
Smallidge,R.L.; Kentzer,E.J.; Stringham,K.R.; Kim,E.H.; Lehe,C.; Stringham,R.W.; Mundell,E.C. Sulfamethazine and sulfathiazole determination at residue levels in swine feeds by reverse-phase liquid chromatography with post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 710–717.

SAMPLE
Matrix: feed
Sample preparation: Weigh out 1 g ground feed, add 3 mL trichloroacetic acid solution, mix well, sonicate at 40° for 10 min, make up to 500 mL with MeCN:10 mM Na_2HPO_4 adjusted to pH 3 with phosphoric acid 20:80, mix well, filter a 500 μL aliquot (Costar spin-X (low type) 0.22 μm cellulose acetate) with centrifuging for 1 min, inject a 10 μL aliquot of the filtrate. (Prepare trichloroacetic acid solution by mixing 87 g trichloroacetic acid with 13 g water, add 0.7 mL of this solution to 99.3 mL acetone.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil-LC-18-DB
Column: 250 × 4.6 5 μm Supelcosil-LC-18-DB
Mobile phase: MeCN containing 0.1% triethylamine:10 mM pH 2.8 Na_2HPO_4 21:79
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 8
Internal standard: sulfamethazine (sulfadimidine)
Limit of quantitation: 250 μg/g

OTHER SUBSTANCES
Simultaneous: sulfadiazine, trimethoprim

KEY WORDS
sulfamethazine is IS

REFERENCE
Hormazabal,V.; Steffanak,I.; Yndestad,M. Simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *648*, 183–186.

SAMPLE
Matrix: feed, premix
Sample preparation: Shake premix or ground feed with 150 mM HCl in MeOH:water 25:75 for 1 h, dilute with 150 mM HCl in MeOH:water 25:75 to achieve a sulfonamide concentration of 5.5 μg/mL, filter (glass fiber), inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2 30-40 μm Perisorb RP-18
Column: 250 × 4.6 10 μm Partisil ODS-3
Mobile phase: MeOH:2% acetic acid 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 450 following post-column reaction. The column effluent mixed with reagent pumped at 0.5 mL/min and the mixture flowed through a 3 m × 0.5 mm ID coil of PTFE tubing to the detector. (Prepare reagent by dissolving 3 g dimethylaminobenzaldehyde in 100 mL glacial acetic acid, add 60 mL MeOH, add 40 mL water, mix well.)

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 1.65 μg/mL

KEY WORDS
post-column reaction

REFERENCE
Stringham,R.W.; Mundell,E.C.; Smallidge,R.L. Use of post-column derivatization in liquid chromatographic determination of sulfamethazine and sulfathiazole in feeds and feed premixes, *J.Assoc.Off.Anal.Chem.*, **1982**, *65*, 823–827.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Suspension + 100 mL MeOH:water 60:40, shake mechanically for 15 min, make up to 200 mL with MeOH:water 60:40, filter (0.45 μm silver membrane, Selas Corp.). Evaporate a 1 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL 200 μg/mL acetanilide in MeCN, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water 20:80
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: acetanilide (11)

OTHER SUBSTANCES
Simultaneous: sulfadiazine, sulfamerazine, sulfanilamide, sulfanilic acid
Noninterfering: erythromycin ethylsuccinate

KEY WORDS
oral suspensions; suspensions

REFERENCE
Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20

Detector: UV 270

CHROMATOGRAM
Retention time: 4.5
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfadiazine, sulfisoxazole, sulfadimethoxine

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 26.6
Limit of detection: 1.7 ppb
Limit of quantitation: 3.6 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 8.5
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole
Interfering: sulfamethizole (distinguish by MS)

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 5.56

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE
Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 PLRPS polymer-based (Polymer Laboratories)
Mobile phase: MeCN:buffer 22.5:80 (Buffer was 7.7 g ammonium acetate, 3 g tetraethylammonium chloride, and 1.8 g EDTA in 1 L water.)
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 8.2

OTHER SUBSTANCES
Simultaneous: sulfapyridine, N-acetylsulfapyridine, sulfasalazine

REFERENCE
Buggé,C.J.; Gautam,S.R.; Parke,L.E.; Mason,J.T.; Garcia,D.B. Simultaneous determination of sulfasalazine and its metabolites sulfapyridine and N-acetylsulfapyridine in human serum by ion-pair high-performance liquid chromatography using a polymer-based column, *J.Pharm.Sci.*, **1990**, *79*, 1095–1098.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfathiazole, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 8.51

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: ethylmorphine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 37.3

OTHER SUBSTANCES
Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfadoxine, sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE
Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Sentry (Waters)
Column: 150 × 4.6 Symmetry C8 (Waters)
Mobile phase: MeOH:water:glacial acetic acid 20:79:1
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: sulfanilamide, sulfadiazine, sulfamerazine, sulfathiazole, succinylsulfathiazole

REFERENCE

Capparella,M.; Foster,W.,III; Larrousse,M.; Phillips,D.J.; Pomfret,A.; Tuvim,Y. Characteristics and applications of a new high-performance liquid chromatography guard column, *J.Chromatogr.A*, **1995**, *691*, 141–150.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: MeCN:50 mM acetic acid 10:90
Flow rate: 2
Detector: UV 266

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: metabolites, acetylsulfamethazine

REFERENCE

Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL 1 mg/mL Solution + 1 mL 100 mM pH 8 sodium bicarbonate + 2 mL 10 mM 1-fluorenylmethyl chloroformate in acetone, let stand for 30 min, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Micro Pak C18 TMS-capped
Mobile phase: Gradient. MeOH:buffer from 35:65 to 85:15 over 4 min (Waters medium concave gradient), maintain at 85:15. (Buffer was 50 mM NaH_2PO_4 adjusted to pH 3.5 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 264 or F ex 264 em 307

CHROMATOGRAM
Retention time: 12.5

KEY WORDS
derivatization

REFERENCE
Liang,G.S.; Zhang,Z.; Baker,W.L.; Cross,R.F. Formation and verification of the structure of the 1-fluorenylmethyl chloroformate derivative of sulfamethazine, *Anal.Chem.*, **1996**, *68*, 86–92.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim
Interfering: sulfamoxole

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 35

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim
Interfering: sulfamoxole

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE

Matrix: tissue
Sample preparation: Cut 20 g tissue into small pieces and add to 25 mL dichloromethane, let stand for 20-30 min with frequent stirring with a glass rod, filter through glass wool, repeat extraction twice more. Combine the dichloromethane layers and shake with 10 mL 1.5 M sulfuric acid for 15 min. Remove the aqueous phase and add it to 10 mL dichloromethane, shake for 10 min, discard the organic phase, repeat the wash, add 1 mL 10% K_2HPO_4 to the aqueous phase, add 3 mL 40% NaOH, mix well, allow to cool, adjust pH to 5-6 with 1.5 M sulfuric acid or 40% NaOH, add 5 mL dichloromethane, extract for 15 min, repeat the extraction. Pass the organic layers through anhydrous sodium sulfate, evaporate to 1 g on a hot plate at 60-70° (in a hood!), inject a 10 μL aliquot. For confirmation of sulfamethazine add 1 mL MeOH to the extract, add 2 drops acetic anhydride, heat at 80-90° on a hot plate until acetic acid fumes are no longer seen, reconstitute with 1 g dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 mm long MicroPak CN-10
Mobile phase: Isooctane:chloroform:MeOH:acetic acid 30.5:65:4:0.5
Flow rate: 0.33
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 4.34 , 5.34 (for acetylsulfamethazine)
Limit of detection: 20 ppb

OTHER SUBSTANCES

Simultaneous: sulfabromomethazine, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfaethoxypridazine
Noninterfering: sulfamerazine, sulfathiazole, sulfanilamide, sulfapyridine, sulfaquinoline

KEY WORDS

cow; liver; fat; kidney; muscle; derivatization

REFERENCE
Seymour,D.; Rupe,B.D. High-pressure liquid chromatographic determination of sulfamethazine residues in beef tissues, *J.Pharm.Sci.*, **1980**, *69*, 701–703.

SAMPLE
Matrix: tissue
Sample preparation: Blend (Waring blender) 10 g tissue and 150 mL chloroform:acetone 50:50 at low speed for 5 min, filter homogenate through 3-4 g Celite (previously washed with 25 mL acetone), rinse blender with acetone, add rinse to filter. Add 10 mL 1 M HCl to the filtrate and evaporate to 5-7 mL under reduced pressure at 40-45°, wash residue with 75 mL hexane. Extract hexane layer twice with 5 mL 1 M HCl. Combine all the aqueous layers and wash with 15 mL dichloromethane. Rinse apparatus with water and add the rinses to the aqueous layer, add 25 mL saturated trisodium citrate to the aqueous layer, adjust pH to 5.8-5.9 with 2 M NaOH, extract the aqueous layer four times with 15 mL portions of dichloromethane. Combine the extracts and evaporate them to dryness under reduced pressure at 40-45°, dissolve residue in 10 mL buffer, add to XAD-2 column, rinse flask three times with 15 mL portions of water, add rinses to column, rinse flask with 100 mL water, add rinse to column, rinse flask with 100 mL MeOH, add rinse to column, collect the MeOH eluate, remove residual MeOH with nitrogen pressure. Add 10 mL 1 M HCl to the eluate, evaporate to dryness under reduced pressure at 40-45°, reconstitute with 8 mL mobile phase, inject a 20 μL aliquot. (Buffer was 3.40 g KH_2PO_4 and 3.55 g Na_2HPO_4 in 1 L water, pH 6.8. Prepare XAD-2 column as follows. Wash (at 60-65 mL/min) 5 kg Amberlite XAD-2 resin in a 1200 × 110 column with water, 3 gallons acetone, 2 gallons MeOH, and 25 gallons water, remove fines by successive decantations, store in water. Prepare a 130 × 15 column of washed resin and wash with 250 mL water. Note that XAD-2 column step is not necessary for fat samples.)

HPLC VARIABLES
Guard column: RP-18
Column: 250 × 4.6 Zorbax ODS
Mobile phase: MeCN:water 25:75
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7-8
Limit of detection: 0.1 ppm

KEY WORDS
pig; liver; kidney; muscle; fat; SPE

REFERENCE
Cox,B.L.; Krzeminski,L.F. High pressure liquid chromatographic determination of sulfamethazine in pork tissue, *J.Assoc.Off.Anal.Chem.*, **1982**, *65*, 1311–1315.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 μL initial mobile phase, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 μL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 20
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

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KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 0.005 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfadiazine, sulfamonomethoxine, sulfadimethoxine, sulfaquinoxaline

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KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue

Sample preparation: Cut tissue into small pieces and homogenize in blender. 20 g Homogenized tissue + 200 μL 10 μg/mL methyl p-aminobenzoate in water + 60 mL acetone:chloroform 50:50, shake vigorously on a mechanical shaker for 10 min, centrifuge at 3000 g for 10 min, filter (Whatman No. 41 paper) supernatant, repeat extraction. Combine the extracts, if the extract is not clear centrifuge at 3000 g for 10 min and discard the aqueous layer, evaporate to an oily residue at 45° under reduced pressure, add 5 mL MeCN to flask, let stand for 10 min, remove MeCN layer, add 5 mL hexane and 5 mL MeCN, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer, add 5 mL MeCN to the hexane layer, shake, centrifuge at 3000 g for 10 min, remove the MeCN layer. If hexane layer is not clear centrifuge at 3000 g for 10 min and remove the clear portion. Add 400 μL 15% trichloroacetic acid to the hexane layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Evaporate the MeCN layers, transfer the oily residue to a small flask with 3 mL hexane, add the aqueous trichloroacetic acid layer, shake gently for 10 min, centrifuge at 3000 g for 10 min. Discard the hexane layer, add 100 μL saturated aqueous sodium citrate solution to the aqueous layer, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP 18 (Brownlee)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 1% aqueous acetic acid. B was MeCN:water 80:20. A:B from 90:10 to 60:40 over 20 min, return to initial conditions over 5 min, re-equilibrate for 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 7 m × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 1 g p-dimethylaminobenzaldehyde in 30 mL MeCN, make up to 100 mL with 5% trichloroacetic acid in water.)

CHROMATOGRAM

Retention time: 14.4
Internal standard: methyl p-aminobenzoate (18.6)
Limit of detection: 20 ng/g

OTHER SUBSTANCES

Extracted: sulfadiazine, sulfamerazine, sulfamethoxypyridazine, sulfapyridine, sulfaquinoxaline

KEY WORDS

chicken; liver; pig; kidney; sheep; cow; post-column reaction

REFERENCE

Bui,L.V. Liquid chromatographic determination of six sulfonamide residues in animal tissues using postcolumn derivatization, *J.AOAC Int.*, **1993**, *76*, 966–976.

SAMPLE

Matrix: tissue
Sample preparation: Extract with supercritical carbon dioxide into a MeOH solution.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 4.5 with phosphoric acid 28:72
Flow rate: 0.5
Detector: UV 270

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: sulfamerazine, sulfamethoxazole, sulfamethizole, sulfamethoxypyridazine, N^4-acetylsulfamethoxazole

KEY WORDS

chicken; pig; liver; muscle; SFE

REFERENCE
Cross,R.F.; Ezzell,J.L.; Richter,B.E. The supercritical fluid extraction of polar drugs (sulphonamides) from inert matrices and meat animal products, *J.Chromatogr.Sci.*, **1993**, *31*, 162–169.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 6
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole
Interfering: sulfamethoxypyridazine

KEY WORDS
post-column reaction; muscle; liver; kidney; SPE

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the

extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM
Retention time: 6.0
Internal standard: sulfaethoxypyridazine (12.8)
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS
cow; pig; muscle; liver; SPE

REFERENCE
Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot.)Sodium acetate should be a highly pure grade.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 10
Limit of detection: 4 ng/g

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfadimethoxine, sulfaquinoxaline

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 9.5
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 11

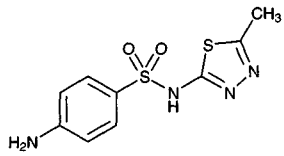
Internal standard: niacin (3.3)

OTHER SUBSTANCES
Extracted: sulfathiazole, sulfacetamide, sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamonomethoxine

KEY WORDS
wastewater

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethizole



Molecular formula: $C_9H_{10}N_4O_2S_2$
Molecular weight: 270.34
CAS Registry No.: 144-82-1
Merck Index: 9084
Lednicer No.: 1 125

SAMPLE
Matrix: formulations
Sample preparation: Dissolve a capsule in 1 L 100 mM HCl, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax TMS
Mobile phase: DMF:water 10:90 containing 5 mM sodium ethylenediaminetetraacetate, 100 mM citric acid, 20 mM sodium citrate, and 50 mM potassium nitrate.
Flow rate: 1.8
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 6.0

OTHER SUBSTANCES
Simultaneous: tetracycline
Noninterfering: phenazopyridine

KEY WORDS
capsules

REFERENCE
Du Preez,J.L.; Botha,S.A.; Lötter,A.P. High-performance liquid chromatographic determination of phenazopyridine hydrochloride, tetracycline hydrochloride and sulphamethizole in combination, *J.Chromatogr.*, **1985**, *333*, 249–252.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or pills. Weigh out an amount of powdered tablets or pills or capsule contents, dissolve in 5 mL EtOH, dilute with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Dilute suspensions or drops with 150 mM HCl containing 40 mM sodium dodecyl sulfate. Filter solutions if necessary. 10 mL Solution in 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 μL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject an aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 μm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

CHROMATOGRAM
Retention time: 5
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfadiazine, sulfaguanidine, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfathiazole
Noninterfering: benzocaine

KEY WORDS
tablets; pills; capsules; suspensions; drops; derivatization

REFERENCE
Garcia-Alvarez-Coque,M.C.; Simo-Alfonso,E.F.; Ramis-Ramos,G.; Esteve-Romero,J.S. High-performance micellar liquid chromatography determination of sulphonamides in pharmaceuticals after azodye precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 237–245.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 12:88
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 21.9
Limit of detection: 1.8 ppb
Limit of quantitation: 3.9 ppb

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamerazine, sulfamethazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE

Matrix: milk

Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2

Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.

Flow rate: 1

Injection volume: 50-500

Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM

Retention time: 8.4

Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

Interfering: sulfamethazine (distinguish by MS)

KEY WORDS

cow; column-switching

REFERENCE

Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE

Matrix: solutions

Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:water:acetic acid 12.5:86.5:1

Flow rate: 1.6

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 9.5

OTHER SUBSTANCES

Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: sulfanilic acid, sulfanilamide, sulfapyridine, sulfamerazine, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfisoxazole, sulfachlorpyridine

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:110 mM perchloric acid and 20 mM sodium acetate 27:73. B was MeCN:110 mM perchloric acid and 100 mM sodium acetate 50:50. A:B from 100:0 to 0:100 over 5 min, then re-equilibrate.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfanilic acid, sulfathiazole, sulfisoxazole

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM
Retention time: 10.77

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 36

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES

Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS

capillary HPLC

REFERENCE

Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 5 µm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.8

Injection volume: 50

Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM

Retention time: 18

Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES

Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS

post-column reaction; muscle; kidney; SPE

REFERENCE

Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Extract with supercritical carbon dioxide into a MeOH solution.

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 4.5 with phosphoric acid 28:72
Flow rate: 0.5
Detector: UV 270

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: sulfamerazine, sulfamethoxazole, sulfamethazine, sulfamethoxypyridazine, N^4-acetylsulfamethoxazole

KEY WORDS
chicken; pig; liver; muscle; SFE

REFERENCE
Cross,R.F.; Ezzell,J.L.; Richter,B.E. The supercritical fluid extraction of polar drugs (sulphonamides) from inert matrices and meat animal products, *J.Chromatogr.Sci.*, **1993**, *31*, 162–169.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 10 mL 150 mM HCl containing 40 mM sodium dodecyl sulfate + 1 mL 100 mM sodium nitrite, let stand for 5 min, add 1 mL 300 mM sulfamic acid, let stand for 10 min, add 500 µL 30 mM N-(1-naphthyl)ethylenediamine dihydrochloride, make up to 25 mL with water, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 35 × 4.6 C18 (Scharlau)
Column: 125 × 4.6 5 µm Spherisorb ODS-2 C18
Mobile phase: Pentanol:50 mM sodium dodecyl sulfate 2.4:97.6, pH adjusted to 7 with 100 mM phosphate buffer
Flow rate: 1
Injection volume: 20
Detector: UV 490

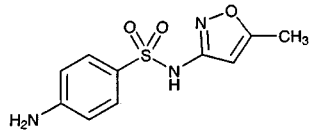
CHROMATOGRAM
Retention time: 5.3
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfaguanidine, sulfamethoxazole, sulfathiazole

KEY WORDS
derivatization

REFERENCE
Simó-Alfonso,E.F.; Ramis-Ramos,G.; García-Alvarez-Coque,M.C.; Esteve-Romero,J.S. Determination of sulphonamides in human urine by azo dye precolumn derivatization and micellar liquid chromatography, *J.Chromatogr.B*, **1995**, *670*, 183–187.

Sulfamethoxazole



Molecular formula: $C_{10}H_{11}N_3O_3S$
Molecular weight: 253.28
CAS Registry No.: 723-46-6
Merck Index: 9086

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 µL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 µm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.42